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Structural Thermodynamics and Kinetics of the Cardiac Myosin/Omecamtiv Mecarbil Complex Revealed by Time-Resolved FRET

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We are investigating the structural thermodynamics and kinetics of cardiac myosin in the presence of omecamtiv mecarbil (OM), a small molecule that is currently in clinical trials for treating heart failure. AM has been shown to increase thin-filament activated cardiac myosin ATPase activity, the apparent rate constant for actin-activated phosphate release from cardiac myosin, and contractility in the heart (Malik et al, Science 2011). How OM modulates theses activities remains poorly understood. Based on a published Biophysical Society Annual Meeting poster abstract (Liu et al. Biophys. J. 2013) and on published studies (Malik et al, Science 2011), we hypothesized that OM stabilizes the pre-powerstroke structural state in the presence of ATP analogs and increases the observed rate constant for the actin-activated powerstroke during actin-activated single ATP turnover. To test these predictions, we used a time-resolved FRET biosensor of β-cardiac myosin, which detects the mole faction of pre and post-powerstroke structural states of the myosin light-chain binding domain, to determine the apparent equilibrium constant for the recovery-stroke under saturating ADP, ADP.BeF, ADP.AlF and ADP.V nucleotide states in the presence of saturating OM or DMSO. We varied the temperatures of these experiments to determine whether OM changes the structural thermodynamics of the pre-powerstroke state. In a second set of experiments, we directly measure light-chain domain rotation during ATP binding to myosin and during actin binding to myosin in the presence of ATP. Taken together, these results address two of the key phenotypes that the drug exhibits in vitro, increased actin-activated phosphate release, and increased thin-filament activated steady-state ATPase cycling.

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Impact of Omecamtiv Mecarbil on Human β-Cardiac Myosin Structure and Function

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Contractile dysfunction is a major problem in systolic heart failure and beta-cardiac myosin provides the driving force for ventricular contraction in humans. The small molecule drug Omecamtiv Mecarbil (OM) specifically targets cardiac myosin and is known to enhance cardiac muscle force generation, while it is unclear how it impacts the structural properties of this motor. We expressed and purified human β-cardiac myosin subfragment 1 (M2B-S1) by generating recombinant adenovirus and infecting C2C12 embryonic muscle cells. We demonstrate that the maximum actin-activated ATPase activity of M2B-S1 is slowed 7-fold in the presence of 10 μM OM, while the actin concentration at which the ATPase activity is one-half maximal (K_{ATPase}) was reduced dramatically (15-fold). The ATPase activity was less ionic strength dependent in the presence of OM. We observed a concentration-dependent inhibition of actin filament sliding velocity in the *in vitro* motility assay. We generated another M2B-S1 construct that contained a tetracysteine site in the upper 50 kDa domain (U50), which enables site-specific labeling of the fluorescein biarscenical hairpin-binding dye (FlAsH). The conformation of the U50 domain was examined by fluorescence resonance energy transfer (FRET) as a function of temperature, using deacADP bound to the active site (donor) and U50 FlAsH (acceptor). When comparing the low (10°C) and high (35°C) temperature FRET, the presence of OM resulted in an increase in the range distances, suggesting the U50 domain is more flexible in the presence of OM. Our results suggest that OM dramatically enhances the affinity for actin which results in an increased duty ratio. The structural impact on the flexibility of the U50 domain may enhance the coupling between the nucleotide and actin binding regions and stabilize the actomyosin.ADP state with high actin and ADP affinity.

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The R146N and R249Q Myosin Mutations Disrupt Motor Function and Myofibrillar Structure and cause Cardiomyopathy in Drosophila

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Numerous myosin-based myopathies have been identified without a detailed understanding of their molecular basis. To pursue mechanistic investigations, we generated transgenic Drosophila expressing the R146N or R249Q myosin mutations that cause hypertrophic cardiomyopathy in humans. Our studies show severely compromised flight ability in homozygotes, with an age-dependent worsening of muscle function and myofibrillar disarray in the indirect flight muscle ultrastructure. The R146N mutation caused an increase in basal Ca-ATPase and Mg-ATPase activities, with a significant decrease in actin motility. Semi-automated optical heartbeat analysis performed using high-speed movies of semi-intact mutant heterozygous hearts indicated restrictive cardiac physiology and diastolic dysfunction. Based upon molecular modeling, we predict 1) the R146N mutation alters ionic interaction of the N-terminal motor domain with E774 of the lever arm, resulting in failure of the lever arm to cock prior to the power-stroke; and 2) the R249Q mutation disrupts electrostatic interaction with D262 of the central β-sheet domain, interrupting communication between the ATP-binding and actin-binding sites. Thus, the models imply that these conserved charge interactions are critical for myosin function. To test these predictions, lines expressing R146E, E774R, R249D and D262R mutations, as well as putative suppressor lines R146E+E774R and R249D+D262R, will be studied using an integrative biochemical, biophysical and physiological approach. Transgenic Drosophila expressing either R146E or R249D myosin revealed more severe phenotypes relative to cardiomyopathy mutations. Both homozygous mutants displayed flightless phenotypes and highly compromised myofibrillar ultrastructure, with the R146E mutants showing increased basal Ca-ATPase and Mg-ATPase activities, and no actin motility. Heterozygotes present with severe restricted cardiac physiology and diastolic dysfunction. Overall our studies indicate that the Drosophila is a valuable tool to test significant myosin interactions during health and disease. Supported by NIH R01GM32443 to SIB.

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Effects of Hypertrophic Cardiomyopathy Causing R403Q Mutation on Human Beta-Cardiac Myosin Biomechanical Function: Single Molecule to Ensemble Studies

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Over 300 known mutations in human beta-cardiac myosin, the motor that powers ventricular contraction, causes familial hypertrophic cardiomyopathy (HCM) by altering the functional capacity of the cardiac sarcomere. The precise mechanism(s) by which these point mutations alter cardiac function and lead to the clinically diverse HCM phenotypes is not well understood. The human heart is optimally designed to cyclically contract under varying loads and it is likely that the mutations in beta-cardiac myosin affect the power output of the heart by either altering force, velocity of contraction, or both, which ultimately results in the observed clinical phenotypes.
Using ensemble in vitro motility experiments and single molecule force spectroscopy, we show that a truncated version (1-808 aa, containing human ELC) of human beta-cardiac HCM-causing R403Q myosin mutant has ~15% lower unitary force generated by a single head but has ~15% higher unloaded velocity of contraction with pure actin. However, when studied with the six-component regulatory thin filament (RTF) system (containing tropomyosin and the troponin complex) the unloaded velocity of R403Q is unchanged. Loaded in vitro motility measurements with both pure actin and RTFs indicate lower ensemble force and therefore possibly lower power generated by this molecule. Additional data suggests that R403Q myosin may have lower affinity for RTFs than WT myosin, signifying a change in the duty ratio. Current efforts exploring the effects of the R403Q mutation with a two-headed HMM construct are underway.

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Characterization of α-Tropomyosin (TM) Mutants that Cause Hypertrophic Cardiomyopathy (HCM) in Humans: In vitro Motility Assays with a Microscopic Heat Pulse

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HCM is characterized by thickening of left ventricular wall and interventricular septum, with abnormalities in contractile functions. It has been thought that Tm's flexible association with actin is important for its function. With in vitro motility assays, we investigated α-Tm mutants V95A and D175N (based on human sequence), which are known to cause HCM. Reconstituted thin filaments with rabbit skeletal actin and Tn were placed on HMM-coated glass surface. At the room temperature (24°C), we applied a microscopic heat pulse by focused